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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Hermona Sorcq et al.

Serial No.: CPA of 09/810,688 Examiner: Deborah Crouch

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For : TRANSGENIC ANIMAL ASSAY SYSTEM FOR ANTI-CHOLINESTERASE SUBSTANCES

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DRAFT

Assistant Commissioner for Patents
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SIR:

DECLARATION UNDER 37 C.F.R. §1.132

I, Hermona Soreq, hereby declare that:

1. I am one of the inventors of the invention set forth in the above-identified patent application.

2. I am a biochemist and expert in the field of Transgenic Animal Assay System for Anti-Cholinesterase Substances. Attached hereto as **Exhibit A** is my curriculum vitae.

3. I have reviewed the subject patent application, which I have been advised is a continuation-in-part of U.S. Serial No. 08/370,156, filed January 9, 1995, now U.S. Patent No. 5,932,780, issued August 3, 1999, which is a

continuation-in-part of U.S. Serial No. 08/202,755, filed February 28, 1994. I have also reviewed the pending claims and the June 28, 2001 Final Office Action, in which the Examiner rejected claims 11-14, 17-20 and 23-25 under 35 U.S.C. §112, first paragraph, for lack of enablement for the preparation and use of transgenic animals comprising any and all variants of said cholinesterase gene. Furthermore, I am aware of the state of the art prior to February 28, 1994 regarding (i) the preparation and use of transgenic non-human animals comprising cholinesterase genes and variants thereof; (ii) the use of such transgenic animals as assay systems; and (iii) the use of such transgenic animals for the production of protein in the milk produced by the mammary glands of the transgenic animal.

4. I understand the issues raised by the Examiner in the June 28, 2001 Final Office Action to be the following:

(A) First, whether the specification provides sufficient description to allow one skilled in the art to make and use transgenic animals comprising any and all variants of the cholinesterase genes or assay systems of these animals. In my opinion, the subject specification does allow one skilled in the art to make and use any transgenic non-human animal bearing hAChE or any variant thereof, and to use such transgenic animals as assay systems and/or for the production of ChE in mammary glands.

(B) Second, whether AChE is a secretory extracellular protein and whether the membrane structure or glycosylation is needed for esterase activity. In my opinion, AChe is a secretory extracellular protein, which does not

require either membrane structure or glycosylation for esterase activity.

(C) Lastly, whether AChE must be catalytically active, and promote neurite outgrowth or neuronal differentiation in order to be enabled. In my opinion, transgenic animals bearing variants of hAChE present a number of phenotypes beyond changes in neuromuscular junction structure (e.g. the "corkscrew-like" neuronal processes), as detailed below.

5. In support of my opinion (A) above, I point out that the subject specification teaches how to make and use two species of transgenic animals, namely, mouse and *Xenopus*. Based on this disclosure, one of skill in the art at the time the subject application was filed, would have reasonably expected that the elements required to express the transgene are conserved across species and therefore, any non-human animal could be prepared and used as a transgenic non-human animal bearing hAChE or its variants. The phenotype of the transgenic animal depends on both the transgene and its activities and the animal into which it is introduced. Nevertheless, there are primary capacities of genes which dictate such phenotypes in any organism, and other, secondary in nature, which can be diverse, depending on the organism. In my laboratory, we have shown over the years that AChE-S overexpression confers a large variety of phenotypes that are characteristic of stress responses, in different tissues of animals and including chemical, psychological and physical stressors. Contrary to the Examiner's cited references, e.g. Mullins (1993 and 1996), applicants have given sufficient description of the integration of the hAChE transgene or its variants into different species and described both the primary and secondary capacities dictating the phenotype to enable one skilled in the art to make any

transgenic animal comprising the cholinesterase gene or its variants.

Furthermore, I have shown how to make and use variants of AChE in the subject application and in related applications on which I am a named inventor. For example, the following AChE variants have been disclosed: i) AChE-I4/E5 in U.S. Serial No. 08/202,755, filed February 28, 1994; ii) AChE-E6 in U.S. Serial No. 08/202,755, filed February 28, 1994, and in the subject application; and iii) both AChE-E6 and I4-AChE in the subject application. I note that the subject application is a continuation-in-part of U.S. Serial No. 08/202,755, filed February 28, 1994. These documents, as well as later publications discussed below, show how to use the transgenic animals as an assay system for anti-cholinesterase substance, as an assay system for stress responses and as an assay system for drug responses.

In addition, AChE transgenic animals can be used for the production of AChE in vivo. For example, the AChE transgenic animal can be used for production of human AChE in mammalian milk, as described in the subject application in Example 10 on pages 112-116.

6. In support of my opinion (B) above, I point out that both the '780 patent and the subject application have disclosed transgenic animals having active AChE produced by the methods described therein. See, for example, column 33, lines 50-64 in the '780 patent and Example 10, starting on page 112 of the subject application.

In addition, numerous publications describing the activity and degree of

glycosylation of hAChE were available prior to the filing date of the subject application, e.g.:

(a) prior art was available describing how secreted acetylcholinesterase can affect neuronal function [Appleyard, M.E. (1992) Trends Neurosci. 15(12):485-90 (copy attached hereto as **Exhibit D**)];

(b) prior art was available demonstrating that abrogation of N-glycosylation in human Acetylcholinesterase has no detectable effect on its enzymatic activity [Velan, B. et al. (1993) Biochem J. 295(Pt3):649-656 (copy attached hereto as **Exhibit E**)].

Further evidence for my opinion is that AChE produced in E. coli is known to be catalytically active (see, Fischer, M. A. Ittah et al.,(1995) "Recombinant human acetylcholinesterase expressed in Escherichia coli refolding, purification, purification and characterization," Biotechnol. Appl. Biochem. 21 (Pt 3): 295-311; and Fischer, M. A. Ittah et al., (1993) 'Expression and reconstitution of biologically active human acetylcholinesterase from Escherichia coli" Cell. Mol. Neurobiol. 13(1): 25-38).

7. In support of my opinion (C) above, a number of phenotypes have been taught for transgenic animals comprising AChE or variants thereof, for example:

(a) lower variability in the bone marrow composition of transgenic mice, described on page 79, lines 12-24 of the subject application (see also U.S. Patent No. 5,932,780, issued August 3, 1999 (hereinafter "'780 patent") at column 35,

lines 18-35);

(b) lower proliferative capacity of hematopoietic cells, described on page 81, lines 1-26 of the subject application (see also '780 patent at column 36, lines 4-25);

(c) resistance to low DFP doses, described on page 92, lines 21-25 of the subject application (see also '780 patent at column 41, lines 14-18);

(d) impaired learning and memory, described on page 98, line 29 to page 94, line 11 of the subject application (see also '780 patent at column 41, lines 58-67); and

(e) induction of neuropathological changes by both catalytically active and inactive AChE transgenes, described in Example 9 on pages 111-112 of the subject application.

Later studies conducted in my laboratory showed the existence of additional phenotypes, such as:

(a) anorexic behavior in AChE-S transgenic mice (copy of an abstract is attached hereto as **Exhibit B**);

(b) stereotypic behavior, a kind of behavior that is associated with schizophrenia and autism in humans, in AChE-R transgenic mice (copy of an abstract is attached hereto as **Exhibit C**).

Furthermore, it has been shown that cholinesterases display morphogenetic properties in various tissues, especially during embryogenesis. This has been described in publications from my laboratory, e.g. Ben-Aziz et al. 1993 (muscle) (copy attached hereto as **Exhibit F**), Soreq et al. 1994 (blood) (copy attached hereto as **Exhibit G**), Karpel et al. 1994 (tumors) (copy attached hereto as **Exhibit H**), as well as by others, e.g. Layer et al. 1993 (neurite outgrowth) (copy attached hereto as **Exhibit I**).

Still further, we have shown that intestinal overproduction of AChE-R is conspicuously higher in AChE-S transgenic mice, but under lethal doses of DFP these mice failed to induce the overproduction response that is characteristic of control mice (Shapira et al. (2000), copy attached as **Exhibit J**); transgenic frogs express muscle tissue pathologies (Seidman et al. (1994), copy attached as **Exhibit K**, and so do the transgenic mice, and the pathology is not limited to the neuromuscular junction (Lev-Lehman (2000), copy attached as **Exhibit L**); in the brain of the transgenic animal, we saw more heat shock protein in the neurons than normal mice show, but AChE-R transgenic mice show less of it, reflecting variant specificity (Sternfeld et al. (2000), copy attached as **Exhibit M**); and Erb et al. measured levels of anxiety in transgenic mice (copy attached as **Exhibit N**). The mice overexpressing the catalytically inactive AChE-S also show S-specific pathologies, implying that at least part of these phenotypes are not due to the catalytic activity of the protein.

8. In my opinion, in view of the methods and examples publicly available as of the priority date of the subject application, i.e. February 28, 1994,

1994, and the teachings set forth in the subject application, one of skill in the art would have been able, without undue experimentation, to: (i) prepare and use any transgenic non-human animal comprising cholinesterase genes and any variants thereof; (ii) use such transgenic animals as assay systems; and (iii) use such transgenic animals for the production of hAChE in the milk produced by lactating female animals.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application or any patent issuing thereon.

Dated: 9.10.02 _____

Hermona Soreq